

# **LEAF EXTRACT OF *U. PILULIFERA* DOWNREGULATES THE EXPRESSION OF INOS IN KIDNEYS OF DIABETIC RATS**

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## **Abstract**

Although the extract of *U. pilulifera* has been used since a long time to treat diabetes, but its role in improving the situation of kidneys remains to be well established. The main objective of the present study is to investigate the effects of the extract of *U. pilulifera* on the expression of iNOS in kidneys of diabetic rats. Methodology of the present study included collection of *U. pilulifera* from various places in Jordan, air dried and extracted by Soxhlet cold extractor using absolute methanol as solvent and remained for three consecutive days. Extracted juice was kept in refrigerator at 4°C. Diabetes was induced through administration of alloxan 150 mg/kg body weight intraperitoneally. Study model included 4 groups: control group, diabetic group, diabetic group treated with 1.25 mg/kg body weight, and diabetic group treated with 1.88 mg/kg body weight. Study findings showed increased significant expression of iNOS in diabetic group compared with control group (P 0.000). The extract of *U. pilulifera* significantly decreased the expression of iNOS in diabetic kidneys at both doses used, but more reduction of iNOS was observed using the large dose 1.88 mg/kg of body weight. Taken together, the present study confirmed the findings of other studies in which the expression of iNOS plays a role in diabetic kidney.

Therapeutic potential of *U. pilulifera* in treating diabetes has also been suggested to be mediated in diabetic kidneys through decreasing the expression of iNOS.

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**Keywords:** Diabetes, iNOS, *U. pilulifera*, kidney, downregulation

## **Introduction**

Diabetic nephropathy (DN) is considered as the most prevalent single cause of end-stage renal disease in the United States and Europe (Veelken et al., 2000). DN is characterized by being a long term complication with a prevalence about 30-40% of patients with diabetes mellitus (DM). It has been indicated that DN is associated with increased renal perfusion and glomerular filtration rate (GFR) (Bildirici et al., 2005).

According to Veelken et al (2000), it has been demonstrated through micropuncture studies that renal vasodilation is the cause of diabetic hyperfiltration. It was thought that renal vasodilatation to be due to increased production of nitric oxide (NO) (Craven et al., 1997). NO has several roles in vascular physiology, neurotransmission, inflammation and immune defense systems (Ignarro et al., 1987). Other studies showed that NO can act as a vascular and neural messenger through activating soluble guanylate cyclase which leads to elevated levels of cGMP. The origin of NO is L-arginine. The family of NOS proteins includes three main members neuronal NOS (nNOS), endothelial NOS (eNOS), and the inducible type (iNOS). iNOS has been found to be expressed by several cell types such as macro-phages, vascular smooth muscle cells, and glomerular mesangial cells, which cause the production of huge amounts of NO. Both of endotoxin and cytokines can induce the production of No (Narita et al., 1995).

According to a study of Sharma et al (1995), it has been postulated that diabetes can induce the production of iNOS which, in turn, leads to increased generation of NO and by thus participates to diabetic hyperfiltration and glomerular abnormalities in diabetes.

Several studies have demonstrated that although diabetes is the main risk factor attributing for DN, but it is not responsible for all observed changes in kidneys (Deckert and Poulsen, 1981; Krolewski et al., 1995; Kosugi et al., 2006).

In the kidney, nitric oxide regulates various physiological functions as glomerular capillary blood pressure, glomerular plasma flow and the glomerular ultrafiltration coefficient (Prabhakar, 2001). From a biological point of view, the effects of NO are due to the concentration of NO at the site of action and the specific location where NO is generated (Raij and Baylis, 1995).

It has been indicated that no exact role of iNOS is established in regulation of renal function as well as its involvement in the pathogenesis of diabetic nephropathy is still a point of controversy (Shireen et al., 2009). Several studies have pointed to the expression of iNOS to be mainly in the tubules of normal kidneys (Ashab et al., 1995; Aiello et al., 1997). Other studies pointed to low or undetectable expression of iNOS in normal kidneys (Bank et al., 1996; Heeringa et al., 1998). On the other hand, studies that investigated the expression of iNOS in diabetes have shown conflicted outcomes. Some studies showed the expression of iNOS in diabetic rat kidney (Sugimoto et al., 1999; Cosenzi et al., 2002), while the expression of iNOS in diabetic kidney was not approved by another study (Veelken et al., 2000).

In the present study, the effect of the extract of *Urtica pilulifera* (*U. pilulifera*) on the expression of inducible nitric oxide synthase (iNOS) on kidneys of diabetic rats was explored. Many studies in literature investigated both the effects of diabetes on the expression of iNOS as mentioned previously, and the effects of the extract of *U. pilulifera* on diabetes. Such studies have shown that the extracts of *U. pilulifera* have been used to treat various diseases including Diabetes Mellitus (Kavalali et al., 2003; Lopatkin et al., 2005).

### **Study objectives**

The main objective of the present study is to investigate the effects of the extract of *U. pilulifera* on the expression of iNOS in kidneys of diabetic rats.

### **Study hypothesis**

There is a significant relationship between the effects of the extracts of *U. pilulifera* and the expression of iNOS in kidneys of diabetic rats which may predict a new therapeutic approach to treat diabetic nephropathy.

### **Methodology**

#### **Plant collection and preparation of extraction**

*U. pilulifera* leaves were collected from various areas at Jordan, air-dried in shade well-ventilated area and then ground into fine powder. About 350 g of powder was put in a Soxhlet cold extractor using absolute methanol as solvent and remained for three consecutive days (Sadki et al., 2001). The extract was concentrated to dryness in rotary evaporator under reduced pressure and controlled temperature (45°C) to yield an 11.4% viscous greenish-colored extract. The extract was kept at 4°C in a glass container until use. Wistar rats were used in this study, in which their average weight was 170 g. The conditions in animal house were to place rats in stainless

steel cages under 12 h light/dark cycle throughout the experimental periods. They had access to food (top fed, Sapele) and water ad libitum. The animals were carefully checked and monitored every day for any changes. After determination of lethal dose (LD50), two doses were selected 1.25 g/kg and 1.88 g/kg of body weight. Doses were prepared through dissolving required amount of the viscous extract in 10 mL Tween-20: 0.9% NaCl (1:9, V/V).

### **Diabetic model**

Diabetes was induced depending on alloxan so that rats were injected by alloxan monohydrate "B.O.H chemical LTD England" intraperitoneally at a dose of 150 mg/kg body weight (dissolved in fresh normal saline) to 18 hr fasted rat. Rats were monitored for blood glucose and rats with blood glucose level over 200 mg/ml, were considered diabetic and employed in the study.

Animals were assigned into the following groups:

Group I: control group; Group II: diabetic group; Group III: diabetic treated with 1.25 mg/kg of body weight; Group IV: diabetic treated with 1.88 mg/kg of body weight.

### **Immunohistochemistry**

Immunohistochemical detection of iNOS was performed using commercially available mouse monoclonal antibodies. Immunohistochemical detections of iNOS was demonstrated by using labeled streptavidin biotin LSAB kit, which consists of secondary biotinylated goat anti-mouse antibody and conjugated streptavidin. Horse raddish peroxidase was followed by 3',3'-Diaminobenzidine (DAB) chromogen. Sections were processed for immunohistochemistry using conventional techniques (Khatib, 2013).

### **Immunohistochemical Assessment of Stained Sections**

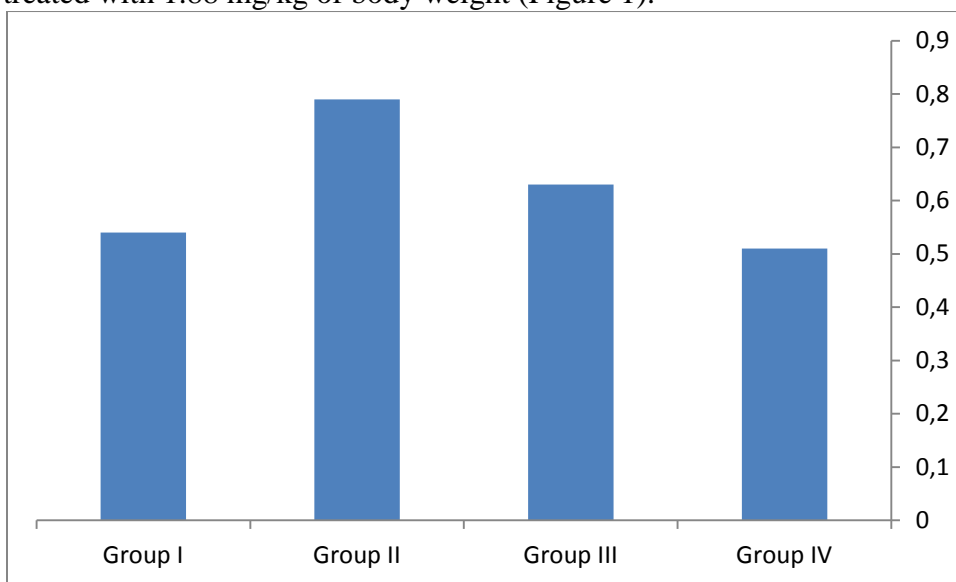
Slides were assessed using adopy photoshop software. Photos for sections were taken and divided into pixels. The total number of pixels was computed and represented both colours (blue and brown), then the brown colour (the colour of the marker under study) was computed and divided by the total number of pixels( Khatib, 2013).

### **Statistical Analysis**

The expression of HSP70 was compared between groups using T test. P value  $\leq 0.05$  was considered statistically significant.

### **Study findings**

After the end of the experiment, the expression of iNOS in kidneys of control group was 0.54. Among diabetic group, the expression of iNOS increased significantly (P 0.000) in kidneys of diabetic rat to 0.79. The effect of the extract of *U. pilulifera* was investigated through two doses, 1.25 mg/kg of body weight and 1.88 mg/kg of body weight. Comparing diabetic group and diabetic group treated with 1.25 mg/kg of body weight, the results revealed significant decreased expression of iNOS in kidneys of diabetic rats from 0.79 to 0.63 respectively (P 0.000). Further significant decreased expression of iNOS (0.51, P 0.000) was noted in kidneys of diabetic rats treated with 1.88 mg/kg of body weight (Figure 1).



**Figure 1: Expression of iNOS among study groups**

## Discussion

The present study investigated the effects of the extract *U. pilulifera* on the expression of iNOS on kidneys of diabetic rats. The results of the present study showed significant increased expression of iNOS in the kidneys of diabetic rats compared with control study. Our findings confirm other studies such as that of Sharma et al (1995), Sugimoto et al (1999) and Cosenzi et al (2002) in which it has been postulated that diabetes can induce the production of iNOS which, in turn, leads to increased generation of NO and by thus participates to diabetic hyperfiltration and glomerular abnormalities in diabetes. However, our results do not agree with other studies in which iNOS has been indicated not to have exact role in regulation of renal function as well as its involvement in the pathogenesis of diabetic nephropathy is still a point of controversy (Shireen et al., 2009). We also do not agree with other researchers who reported low or undetectable expression

of iNOS in normal kidneys (Bank et al., 1996; Heeringa et al., 1998). We also do not agree with the results of Veelken et al (2000) who did not approve the expression of iNOS in diabetic kidney.

It is worth to mention that technical issues may be beyond the detection of iNOS in kidneys and improvements of immunohistochemistry have helped greatly in localization of tissue antigens.

The results of our study showed a significant decreased expression of iNOS in diabetic kidney using either 1.25 mg/kg of body weight or 1.88 mg/kg of body weight. Profound effects were seen using the large dose. These findings agree with other results which showed significant effects of *U. pilulifera* on diabetic persons (Kavalali et al., 2003; Lopatkin et al., 2005). These studies did not show how molecular mechanisms are involved in improving diabetic conditions. The importance of the present studies comes through opening new doors for understanding the effects of herbal treatments.

## Conclusion

The present study confirmed the findings of other studies in which the expression of iNOS plays a role in diabetic kidney. Therapeutic potential of *U. pilulifera* in treating diabetes has also been suggested to be mediated in diabetic kidneys through decreasing the expression of iNOS.

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